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(54) Title: DES-TYR DYNORPHIN ANALOGUES

(57) Abstract

Novel peptides of the invention are dynorphin analogues and have similar activity to endogenous dynorphin, but are des-Tyr or des-Tyr-Gly with respect to endogenous dynorphin. The novel peptides have therapeutic uses, such as administration to a host tolerant to a narcotic analgesic in order to potentiate activity of the narcotic analgesic and/or to block withdrawal symptoms.

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# DES-TYR DYNORPHIN ANALOGUES

## Field of the Invention:

The present invention generally relates to dynorphin analogues, and more particularly to novel dynorphin analogues that can be used with narcotic analgesics, such as opiate alkaloids. This invention was made with government support under Grant No. NIDA-02643 awarded by the National Institutes of Health. The Government has certain rights in this invention.

## Background of the Invention:

The endogenous opioids exist in multiple forms in the central nervous system and include the dynorphins, which are a series of peptides derived from the precursor prodynorphin (proenkephalin B). The first of the dynorphins to be isolated was the 17 amino acid peptide having the structure shown (and designated SEQ ID NO:1), sometimes also referred to as "dynorphin A (1-17)":

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln (SEQ ID NO:1)

Unlike either the enkephalins or th end rphins, many of th dynorphins interact with high affinity with all three major opioid receptor typ s ($\mu$, $\delta$, and $\kappa$). The dyn rphins are also nearly unique among endogen us

opioids in that they are not analgesic in the brain, although they may be in the spinal cord.

Smith and Lee have recently reviewed the pharmacology of dynorphin in *Ann. Rev. Pharmacol. Toxicol.*, *28*, pp. 123-140 (1988). They note a growing body of evidence has indicated that endogenous opioids are closely connected with function of the immune system. The reviewers, however, state that dynorphin had not been tested in any of the studies reviewed, except for one study concerning mononuclear cell chemotaxis. However, the reviewers note that dynorphin has been implicated in tumor formation.

Several U.S. patents have suggested uses of dynorphin. U.S. Patent 4,361,553, issued November 30, 1982, inventors Loh and Lee, set out the sequence of the first thirteen peptides for the naturally occurring dynorphin (containing seventeen amino acids), which had been discovered to have potent agonist properties in guinea pig ileum and mouse vas deferens. This patent describes the discovery that dynorphin, and particularly dynorphin A (1-13) has an opposite effect in hosts tolerant to narcotic analgesic than the effect which has been observed in naive animals (an inhibition of morphine or ß-endorphin-induced analgesia). Thus, dynorphin A (1-13) potentiates the analgesic effect in tolerant hosts. Dynorphin was found useful in conjunction with a narcotic analgesic in order to reduce the amount of narcotic analgesic administered per dose.

U.S. Patent 4,396,606, issued August 2, 1983, inventor Goldstein, describes isolation of a compound referred to as "dynorphin" (sometimes hereinafter called "dynorphin (1-13)") with the structure:

Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:2)

This compound was found to be substantially more active than the enkephalins and ß-endorphin in a guinea pig ileum test, and compositions containing the compound were suggested to be analgesic.

U.S. Patent 4,462,941, issued July 31, 1984, inventors Lee et al., describes dynorphin amide analogs with the first seven amino acids as in SEQ ID NO:1 and SEQ ID NO:2, but with the next several amino acids as:

$$AA^8—AA^9—AA^{10}$$

wherein $AA^8$ is isoleucine, leucine, or lysine, $AA^9$ is arginine or proline, $AA^{10}$ is proline, and a carbonyl carbon at the $AA^{10}$ terminus is amidated. These dynorphin (1-10) amide analogs do not have significant analgesic activity (unless given in huge doses where they tend to produce convulsions), but they differ from the SEQ ID NO:2, dynorphin (1-13) by neither potentiating nor antagonizing morphine in naive animals. In tolerant animals, on the other hand, the dynorphin (1-10) amide analogs appear to be a more potent and selective analog than dynorphin (1-13). SEQ ID NO:3 represents a particular one of these dynorphin (1-10) amide analogs (where the C-terminal Pro is amidated):

Tyr—Gly—Gly—Phe—Leu—Arg—Arg—Ile—Arg—Pro (SEQ ID NO:3)

U.S. Patent 4,481,191, issued November 6, 1984, inventors Wei et al., describe a method for treating high blood pressure and disturbances of cardiac function by administrating dynorphin-related opioid peptides, such as the SEQ ID NO:2 and SEQ ID NO:3 peptides. It appears that endogenous opioid peptides condition the sensitivity of the peripheral nerves to stimuli that affect heart rate and blood pr ssure.

Thus, circulating opioid peptides, under normal conditions, are operating to control the sensitivity of these peripheral sites of the autonomic nervous system to such endogenous substances. Use of the dynorphin-related peptides in treating high blood pressure appears to modify the autonomic nervous system so as to amplify and maintain the intensity of endogenous opioid peptides. A mode of action may be by increasing the sensitivity of visceral afferent receptors.

Enkephalin analogues that are conformationally constrained by a cyclic structure (such as with a disulfide bridge) are described by U.S. Patent 4,518,711, issued May 21, 1985, inventors Hruby et al. These compounds are said to have increased rigidity and increased delta receptor specificity if the half-cysteine in the 2 position is replaced by half-penicillamine (ß,ß-dimethyl half-cysteine). Subsequently, dynorphin analogues have become known that have cysteine replacements at the amino acid residue 5 (usually leucine) and at the amino acid residue 11 (usually lysine). The amino acid residue 8 (usually an isoleucine) and the amino acid residue 13 (usually a lysine) have similarly been replaced by cysteines in a bridged relationship. The bridges, or cyclic structures, appear to assist in stabilizing the dynorphin analogues against *in vivo* degradations.

U.S. Patent 4,684,624, issued August 4, 1987, inventors Hosobuchi et al., describe the use of dynorphin-related peptides, in the acid or amidated form, to treat patients suffering from cerebral ischemia. The administration of these opioid peptides to patients suffering from acute focal cerebral ischemia has been found useful in prolonging survival, and appears useful in partially reversing neurologic deficits resulting from cerebral isch mia.

Investigators have recently begun attempts to link immuno regulation to neural opioid systems. It has become increasingly clear there are a number of opioid effects on cells of the immune system, but the mechanisms remain obscure. The authors of a recent review have concluded that the significance of opioids in immune system function remain a matter for speculation. Sibinga and Goldstein, *Ann. Rev. Immunol.*, *6*, pp. 219-249 (1988).

In 1991, Roy et al. reported that mice having been treated with chronic morphine were immuno-suppressed, whereas use of either SEQ ID NO:2 or SEQ ID NO:3 was found to block the opioid inhibition of macrophage-colony stimulating factor of morphine in a dose-dependent manner. *Eur. J. of Pharm.*, *195*, 359-363 (1991); *202*, 355-359 (1991).

Summary of the Invention:

In one aspect of the present invention, novel peptides are described that have at least six amino acid residues, are analogues of dynorphin, but are des-Tyr with respect to the endogenous dynorphin. These novel peptides may be formulated in a pharmaceutically acceptable solution or with a pharmaceutically acceptable carrier, and are usefully administered to a host tolerant to a narcotic analgesic in order to potentiate activity of the narcotic analgesic and/or to block withdrawal symptoms. Additional uses include the reversal of at least some neurologic deficit in treating cerebral and spinal ischemia, in inhibiting respiratory depression or gastroenteric spasms produced by narcotic analgesics to a naive host, as an adjunct for anti-inflammat ry medication, and in blocking narcotic induced immune impairment in a host whose immune system has been impair d by a narcotic analgesic.

Thus, novel peptides of the invention generally have similar activity to endogenous dynorphin (SEQ ID NO:1), to dynorphin with thirteen amino acids (SEQ ID NO:2), and to dynorphin in amide form with ten amino acids (SEQ ID NO:3); however, it is surprising that the novel compounds, which are des-Tyr with respect to such previously known dynorphin compounds, exhibit similar biological activity because the N-terminal tyrosine has been considered a substantially universal requirement for recognition of opioid peptides by opioid receptors. For example, researchers in the field seeking to clone cDNA encoding an opioid receptor have recently noted that the des-Tyr Dyn A (1-13) did not compete at all in assays with various ligands (pp. 4126 and 4127-4128), and recited the conventional wisdom concerning the "necessity" of the N-terminal Tyr. Xie, Miyajima, and Goldstein, *Proc. Natl. Acad. Sci. USA, 89*, pp. 4124-4128 (1992).

In the therapeutic uses of this invention that include administration to a host tolerant to a narcotic analgesic, lower doses of the narcotic analgesic, for example an opiate alkaloid such as morphine, may be used for patients requiring chronic treatment with narcotics to ease pain, such as terminal cancer patients, or lower doses of a narcotic such as methadone may be used in treating narcotics addicts. As a consequence, the various, known side effects, such as respiratory depression and constipation, which result from chronic treatment with high doses of narcotics, can be lessened by practice of the invention.

Detailed Description of the Embodiment:

Novel peptides of the invention have at least six amino acids for the various desirable therapeutic applications. Where the at 1 ast six amino acid

residues are present, then they preferably are in the sequence: Gly-Phe-Leu-Arg-Arg-Ile (SEQ ID NO:22).

In one aspect of this invention, novel peptides can be viewed as having amino acid residues analogous to endogenous dynorphin (SEQ ID NO:1), but where the novel peptides are des-Tyr, as shown by SEQ ID NOS:4-12:

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn (SEQ ID NO:4);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp (SEQ ID NO:5);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp (SEQ ID NO:6);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:7);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu (SEQ ID NO:8);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys (SEQ ID NO:9);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro (SEQ ID NO:10);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg (SEQ ID NO:11); and,

Gly-Gly-Phe-Leu-Arg-Arg-Ile (SEQ ID NO:12).

Further, in any of the novel SEQ ID NOS:4-12, any one or two of the residues may be replaced with the same or a different amino acid residue in the D-configuration (to increase *in vivo* stability), such as where the N-terminal Gly is replaced by D-Ala, or a modification for conformational stability or rigidity may be made, such as where a plurality of the specified amino acid residue are replaced by moieties capable of forming a cyclic structure, or bridge (e.g., the disulfid bridge).

Nov 1 peptides for this invention can also be viewed as des-Tyr-Gly, as shown by SEQ ID NOS:13-22,

which can similarly be modified to increase *in vivo* stability and conformational stability as already described for SEQ ID NOS:4-12:

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln (SEQ ID NO:13);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn (SEQ ID NO:14);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp (SEQ ID NO:15);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp (SEQ ID NO:16);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:17);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu (SEQ ID NO:18);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys (SEQ ID NO:19);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro (SEQ ID NO:20)

Gly-Phe-Leu-Arg-Arg-Ile-Arg (SEQ ID NO:21); and,

Gly-Phe-Leu-Arg-Arg-Ile (SEQ ID NO:22).

The novel peptides illustrated by SEQ ID NOS:1-22 can be in the acid or amide form at the C-terminus. Further, the usual isoleucine amino acid residue in SEQ ID NOS:4-22 can be replaced with leucine or lysine, and the usual arginine at position 8 of SEQ ID NOS:4-11 and at position 7 of SEQ ID NOS:13-21 can be replaced with proline.

One model potentially useful for choosing particular modifications is based upon a prediction that receptor selectivity of opioid peptides is governed by their net charge and/or amphiphilic moment, in addition to the structural and conformational requirements of a particular opioid receptor type ($\mu$, $\delta$, and $\kappa$). This model predicts that opioid peptides with a net positive charge would show $\mu$-receptor preference while neutral and negatively charged opioid peptides would

preferentially interact with the δ-receptor, and the model has been used, for example, in designing small peptides by Schiller et al., *J. of Med. Chem.*, *32*, pp. 698-703 (1989).

Novel peptides of the invention are preferably formulated in a pharmaceutically acceptable solution or with a pharmaceutically acceptable carrier, and then are administered in conjunction with a narcotic analgesic.

Depending upon the mode of administration, the peptide may be formulated with a wide variety of physiologically acceptable carriers, such as aqueous saline and phosphate buffered saline, and may include physiologically acceptable excipients, such as glucose, mannitol, or the like.

In addition to use of the novel peptides in the therapeutic applications described more fully below, we have surprisingly discovered that the dynorphin (1-17), also known as SEQ ID NO:1, but when des-Tyr (and thus described as SEQ ID NO:23), retains sufficient of the desired activity in conjunction with narcotic analgesics to be useful.

The present invention is useful with substantially all narcotic analgesics, and more preferably the opiate alkaloids and opioid peptides (both synthetic and natural). For example, the invention is useful with the various alkaloids of opium such as morphine, morphine salts (such as morphine hydrobromide, morphine hydrochloride, morphine muscate, morphine oleate, morphine N-oxide, and morphine sulfate), and morphine analogs such as normorphine, diacetyldihydromorphine, diacetylmorphine hydrochloride, codeine, and diacetylmorphine (heroin). Other widely used narcotic analgesics with which the present invention may be used include alphaprodine, methadone, meperidin , levorphanol, propoxyphene, fentanyl and its analogues, oxymorphone, anil ridine, dilaudid, and metopon. Uses

can be extended to the peptide analgesics, such as enkephalins and ß-endorphin analogs.

As is well known, continued use of these narcotic analgesics leads to habituation or addiction, and use of one leads to cross-tolerance for the others. However, despite their abuse potential, these narcotic analgesics have therapeutic uses, for example with patients requiring chronic treatment to ease pain.

Even in such therapeutic uses, though, patients develop increasing tolerances to these narcotic analgesics, so that increasingly potent doses are required to achieve relief from pain. Undesirable side effects then tend to develop to the large, chronic doses of the narcotic analgesics.

The agonistic actions and dependence-producing properties of narcotic analgesics can be, and are, studied in various mammalian species besides humans, since practical and governmental considerations frequently require that studies be first done in small rodents and/or monkeys before the analgesic properties of pharmaceuticals are tested with humans. To the present, however, all drugs that have morphine-like properties in mammals other than humans have been found to be morphine-like in humans, and a variety of analgesic assays have been developed with animals which have gained widespread acceptance for predicting properties in humans.

The present invention includes administering a dose of one of the analogues SEQ ID NOS:4-23, or a modified version thereof as has been described, to a host in conjunction with administering a dose of a narcotic analgesic, wherein the administration is within at least about 30 minutes of the narcotic analgesic dose. Pr ferably the administering is by administering a single, admixed dose where the narcotic analgesic, is

morphine, a morphine analogue, or a morphine salt, or other peptide analgesics.

Where administering of narcotic analgesic is morphine and is to a naive patient, a normal dosage is on the order of about 5 mg i.v., assuming a body weight of about 70 kg. It is believed a suitable dose of the dynorphin analogue, administered in conjunction with the analgesic, is from about 30-1500 μg per kg body weight. Although the dynorphin analogue does not potentiate the narcotic analgesic in an initially naive host, as the patient continues in an extended treatment with narcotics to ease pain, the amount of narcotic required to produce a sufficient level of analgesia over the treatment period will be less than without use of dynorphin analogue in conjunction with the narcotic. As a consequence, the various undesirable side effects of repeated, high doses of narcotics, can be lessened.

The dosage in tolerant patients may be determined as follows. A first, or sufficient, dose of the narcotic analgesic is determined which would be sufficient to produce analgesia in the host. However, instead of administering the sufficient dose, a predetermined dose of the narcotic analgesic is administered. This predetermined, or second, dose includes less of the narcotic analgesic than would be sufficient to produce analgesia in the host. The actually administered dose of narcotic analgesic is supplemented with dynorphin analogue. The supplementation is preferably sufficient to produce a level of analgesia in the host which is substantially equivalent to the level of analgesia were solely the narcotic analgesic to have been administered. As may be understood, the first or sufficient dose, the lower, second dose, and the supplementing dose will vary depending upon the patient's particular level f

tolerance to the narcotic analgesic, and will normally be determined by the treatment physician.

Another therapeutic method of use is in treating addicts to substantially block withdrawal symptoms.

Presently, many addicts are placed upon a methadone (usually methadone hydrochloride) maintenance program. In conjunction with the administration of methadone, another drug, such as clonidine, is administered in conjunction therewith. However, as is well known, methadone is itself addictive, and clonidine is believed to simply mask withdrawal symptoms. As a consequence, patients on such programs are not actually being "cured" of their narcotic addiction.

By contrast, dynorphin analogues block the withdrawal symptoms of morphine addicted hosts, yet are at least 100 times less addictive than morphine. Accordingly, the administrating of the present invention may be used to assist in blocking withdrawal symptoms in therapeutic treatments of narcotic addicts being treated for addiction.

Thus, it is believed that administering a dose of dynorphin analogue to a host tolerant to narcotic analgesics will provide a significantly more desirable treatment in treating narcotic addiction.

Novel peptides of the invention can further be used partially to reverse neurologic deficits in cerebral ischemia. It is believed that factors affecting response to therapy for cerebral ischemia in accordance with the present invention include the dosage, the route of administration, and duration of therapy. However, blood pressure does not appear to be a factor affecting response to therapy for cerebral isch mia in accordance with the present invention.

In treating patients suffering from acute focal cerebral ischemia in accordance with the present

invention, therapy is initiated by administering a dose of the dynorphin analogue and then preferably continued by administering subsequent doses.

The initial dose may be from about 1.0 μg/kg of patient's weight to about 10 mg/kg of patient's weight, more preferably about 100 μg/kg of patient';s weight, and can be delivered by various means known to the art, such as by intravenous injection ("I.V."). Subsequent doses may also be delivered by various means known to the art, such as by injections or through topical applications in conjunction with a drug carrier, such as dimethyl sulfoxide. However, it is preferred that the subsequent doses be delivered substantially continuously for as long as the patient is in a life threatening situation, or until the patient's condition stabilizes, and be at a rate between about 0.01 μg/hr to about 100 μg/hr. For example, continuous infusion may be by use of an implanted mini-pump, or by I.V. When the patient's condition stabilizes, then the doses may be gradually reduced, or titrated.

Aspects of the invention will now be illustrated by the following examples.

## EXAMPLE 1

### Methods and Materials

Analgesia was measured by the tail-flick method of D'Amour and Smith, *J. Pharmac. Exp. Ther.*, *72*, pp. 74-79 (1941), incorporated herein by reference, as modified by Tulunay and Takemori, *J. Pharmac. Exp. Ther.*, *190*, pp. 395-400 (1974), incorporated herein by reference. For $ED_{50}$ (e.g., effective does for 50% of the test group) determinations, the animals' responses were made quantal by establishing an endpoint which represents a significant increase in reaction time. The endpoint was an increase in reaction time of an

individual animal of greater than 3 SD (e.g., standard deviation) of the control mean reaction time for all animals used in the assay. The usual control mean reaction time was 3.1±0.05 sec. Nonresponding animals were removed from the heat stimulus when reaction time exceeded 10 sec. to avoid tail damage.

Drugs were injected 30 minutes prior to testing, unless otherwise indicated. Morphine was injected subcutaneously (s.c.) whereas the peptides were injected (i.v.) in 4 ml saline.

Morphine tolerance was established by implanting morphine pellets, 75 mg base, subcutaneously by the method of Way et al., *J. Pharmac. Exp. Ther.*, *167*, pp. 1-8 (1969), incorporated herein by reference. The drug used was morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO). The $ED_{50}$ values, their 95% confidence limits and significance of the potency ratio between two $ED_{50}$ values were determined by the method of Litchfield and Wilcoxon, *J. Pharmac. Exp. Ther.*, *96*, 99-113 (1949), incorporated herein by reference.

Procedure for Table 1 Data:

The morphine tolerant (addicted) animals had pellets implanted for three days. The animals were then challenged with naloxone doses (while the morphine pellet remained in place). The naloxone places the animals into a state of narcotic withdrawal and the animal exhibits withdrawal symptoms because naloxone is an antagonist of morphine. Table 1 summarizes the data for the control animals and for groups of animals treated with three different peptides. Each peptide was in a dose of 5 μmol/kg i.v. befor administration of the nal xone.

TABLE 1

| Peptide Administered | Naloxone $ED_{50}$ (μmol/kg) | Potency Ratio |
|---|---|---|
| (control) | 79 (59-107) | ---- |
| Dyn (2-17) SEQ ID NO:23 | 289 (218-378) | 3.7 (2.5-5.4) |
| [$Cys^5$-$Cys^{11}$] Dyn (1-11) $NH_2$ | 208 (151-279) | 2.6 (1.7-3.9) |
| Dyn (3-13) SEQ ID NO:17 | 154 (102-249) | 2.0 (1.2-3.6) |

As is seen by the data of Table 1, the control animals had an $ED_{50}$ of 79 μmol/kg, which was the base line value. However, when any of the three peptides shown in Table 1 was administered five minutes before administration of the naloxone, then much more naloxone was needed to precipitate the animal into a state of narcotic withdrawal. This means that each of the three peptides shown in Table 1 caused the addicted animal to be not as dependent on morphine as it would be without such pretreatment.

Of the three peptides summarized in Table 1, the dynorphin (3-13), SEQ ID NO:17 is also a novel compound. Although this novel compound is des-Tyr-Gly, it has a potency ratio of 2. That is, addicted animals that were pretreated with this novel peptide before receiving the narcotic antagonist were only half as dependent on morphine as addicted animals which were not so pretreated. Additionally, the cyclic dynorphin amide compound used (where the normal leucine at the 5 position and the normal lysine at the 11 position had been r placed by cysteines, whose disulfide bridge provides conformational stability) had a potency ratio

of 2.6, while the des-Tyr compound surprisingly had a potency ratio of 3.7.

EXAMPLE 2

Materials and Methods:

The animals and test procedures used were analogous to those described in Example 1.

Protocol for Table 2:

The antinociceptive activity ("pain killing") of morphine was tested in morphine tolerant (that is, addicted) mice, as well as in naive, unaddicted (that is, normal) animals. Two base lines were thus established for the two different control animals.

TABLE 2

| Peptide Administered | Morphine $ED_{50}$ ($\mu$mol/kg s.c.) | Tolerance Index |
|---|---|---|
| (control[1] -- naive, unaddicted animals) | 6.5 (5.0-8.9) | ---- |
| (control[2] -- addicted animals) | 55.6 (42.6-73.5) | 8.5 (5.7-12._) |
| (addicted animals) Dyn (2-17) SEQ ID NO:23 | 32.2 (26.7-40.0) | 4.7 (3.5-6.2) |

As is seen from the data of Table 2, the first control (unaddicted) animals had a morphine $ED_{50}$ of 6.5. This means that when the naive animals were administered 6.5 $\mu$mol/kg s.c. morphine before the tail flick test, then half of those animals felt no pain. By contrast,

the second control group, which were morphine addicted animals, required an amount of morphine increased by a factor of 8.5 in order for half of the animals to feel no pain. However, the similarly addicted animals, when pretreated with the des-Tyr compound (at 2.5 μmol/kg i.v. 5 minutes before testing) had a significantly decreased amount of morphine necessary for the pain relief.

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It is to be understood that while the invention has been described above in conjunction with preferred specific embodiments, the description and examples are intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims.

It is Claimed:

1. A peptide having the sequence:
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn (SEQ ID NO:4);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp (SEQ ID NO:5);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp (SEQ ID NO:6);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:7);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu (SEQ ID NO:8);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys (SEQ ID NO:9);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro (SEQ ID NO:10);
Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg (SEQ ID NO:11); and,
Gly-Gly-Phe-Leu-Arg-Arg-Ile (SEQ ID NO:12),
wherein the C-terminus is in acid or amide form.

2. A peptide having the sequence:
Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln (SEQ ID NO:13);
Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn (SEQ ID NO:14);
Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp (SEQ ID NO:15);
Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp (SEQ ID NO:16);
Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:17);
Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu (SEQ ID NO:18);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys (SEQ ID NO:19);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro (SEQ ID NO:20)

Gly-Phe-Leu-Arg-Arg-Ile-Arg (SEQ ID NO:21); and,

Gly-Phe-Leu-Arg-Arg-Ile (SEQ ID NO:22),

wherein the C-terminus is in acid or amide form.

3. The peptide as in claim 1 or 2 wherein one or two of the amino acid residues has been replaced with an amino acid residue in the D-configuration.

4. The peptide as in claim 1 or 2 adapted for therapeutic administration by formulation in a pharmaceutically acceptable solution, or with a pharmaceutically acceptable carrier and/or by a modification to increase conformational or *in vivo* stability.

5. A therapeutic method comprising:

administering a dose of a dynorphin analogue, said dose including at least one peptide having the sequence set out in claim 1, 2 or 3 or adapted as in claim 4, the administering being to a host tolerant to a narcotic analgesic.

6. The therapeutic method as in claim 5 wherein:

the administering of said dose is in conjunction with and within at least about 30 minutes of administering a dose of narcotic analgesic.

7. The therapeutic method as in claim 6 wher in the dos of dynorphin analogue and the dos of narcotic analgesic are substantially simultaneously administered.

8. The therapeutic method as in claim 6 wherein the dose of narcotic analgesic includes an opiate alkaloid or an opioid peptide.

9. The therapeutic method as in claim 6 wherein the narcotic analgesic is morphine, a morphine analogue, or a morphine salt.

10. A therapeutic method for treating a patient tolerant to a narcotic analgesic comprising:

administering a dose of a dynorphin analogue in an amount effective to block narcotic analgesic withdrawal symptoms and/or to potentiate a narcotic analgesic when administered in conjunction therewith, the dynorphin analogue administered being a dynorphin analogue that is des-Tyr or des-Tyr-Gly at the N-terminus.

11. The therapeutic method as in claim 10 wherein the dynorphin analogue is administered in a pharmaceutically acceptable solution.

12. The therapeutic method as in claim 10 wherein the dynorphin analogue has at least six amino acid residues.

13. The therapeutic method as in claim 10 wherein the dynorphin analogue is amidated at the C-terminus.

14. The therapeutic method as in claim 10 wherein the dynorphin analogue has an *in vivo* stability or a conformational stability increased with respect to th amino acid sequence of endogenous dynorphin.

AMENDED CLAIMS

[received by the International Bureau on 13 November 1993 (13.11.93); original claim 12 cancelled; original claims 2-5 and 10 amended; other claims unchanged (3 pages)]

1. A peptide having the sequence:

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn (SEQ ID NO:4);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp (SEQ ID NO:5);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp (SEQ ID NO:6);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:7);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu (SEQ ID NO:8);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys (SEQ ID NO:9);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro (SEQ ID NO:10);

Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg (SEQ ID NO:11); and,

Gly-Gly-Phe-Leu-Arg-Arg-Ile (SEQ ID NO:12),

wherein the C-terminus is in acid or amide form.

2. A peptide having the sequence:

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln (SEQ ID NO:13);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn (SEQ ID NO:14);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp (SEQ ID NO:15);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp (SEQ ID NO:16);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (SEQ ID NO:17);

Gly-Phe-Leu-Arg-Arg-Il -Arg-Pro-Lys-Leu (SEQ ID NO:18);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys (SEQ ID NO:19);

Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro (SEQ ID NO:20); and

Gly-Phe-Leu-Arg-Arg-Ile-Arg (SEQ ID NO:21),

wherein the C-terminus is in acid or amide form.

3. The peptide as in claim 1 having an *in vivo* effect when administered in conjunction with or sequentially to a narcotic analgesic wherein the N-terminal Gly amino acid residue has been replaced with an amino acid residue in the D-configuration to increase *in vivo* stability while retaining said *in vivo* effect.

4. The peptide as in claim 1 or 2 adapted for therapeutic administration by formulation in a pharmaceutically acceptable solution, or with a pharmaceutically acceptable carrier.

5. A therapeutic method comprising:

administering a dose of a dynorphin analogue, said dose including at least one peptide having the sequence set out in claim 1 or 2 or adapted as in claim 4, the administering being to a host tolerant to a narcotic analgesic.

6. The therapeutic method as in claim 5 wherein:

the administering of said dose is in conjunction with and within at least about 30 minutes of administering a dose of narcotic analgesic.

7. Th therap utic method as in claim 6 wher in the dose of dynorphin analogue and the dose f

narcotic analgesic are substantially simultaneously administered.

8. The therapeutic method as in claim 6 wherein the dose of narcotic analgesic includes an opiate alkaloid or an opioid peptide.

9. The therapeutic method as in claim 6 wherein the narcotic analgesic is morphine, a morphine analogue, or a morphine salt.

10. A therapeutic method for treating a patient tolerant to a narcotic analgesic comprising:

administering a dose of a dynorphin analogue in an amount effective to block narcotic analgesic withdrawal symptoms and/or to potentiate a narcotic analgesic when administered in conjunction therewith, the dynorphin analogue administered being a dynorphin analogue that is des-Tyr or des-Tyr-Gly at the N-terminus and has at least seven amino acid residues.

11. The therapeutic method as in claim 10 wherein the dynorphin analogue is administered in a pharmaceutically acceptable solution.

12. (CANCELLED)

13. The therapeutic method as in claim 10 wherein the dynorphin analogue is amidated at the C-terminus.

14. The therapeutic method as in claim 10 wherein the dynorphin analogue has an *in vivo* stability or a conformational stability increased with respect to the amino acid sequence of endogenous dynorphin.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05161

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :A61K 37/00, 37/02; C07K 5/00, 7/00, 15/00, 17/00
US CL :530/326, 327, 328, 329; 514/13, 14, 15, 16, 17
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/326, 327, 328, 329; 514/13, 14, 15, 16, 17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | EUROPEAN JOURNAL OF PHARMACOLOGY, VOL. 153, ISSUED 1988, LONG ET AL., "HINDLIMB PARALYTIC EFFECTS OF PRODYNORPHIN-DERIVED PEPTIDES FOLLOWING SPINAL SUBARACHNOID INJECTION IN RATS", PAGES 45-54, SEE ENTIRE DOCUMENT. | 1-14 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be part of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

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|---|---|
| Date of the actual completion of the international search<br>14 September 1993 | Date of mailing of the international search report<br>SEP 20 1993 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. NOT APPLICABLE | Authorized officer<br>MERRELL CASHION, SPE<br>Telephone No. (703) 308-0196 |

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